

Study of Morphological and Morphometric Characteristics of Worker Bees *Tetragonula clypearis* in North Lombok Regency, West Nusa Tenggara Province, Indonesia

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Article History

Received : July 02th, 2022

Revised : August 20th, 2022

Accepted : September 24th, 2022

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Abstract: This study aimed to describe the morphometric characteristics of the worker bee *Tetragonula clypearis* in Salut Village, District Kayangan, North Lombok Regency. The research material used was worker bee *Tetragonula clypearis* taken from 40 stup in two livestock groups, namely 20 stup in the Harapan Keluarga group and 20 stup in the Tunas Muda group in Salut Village, Kayangan District, North Lombok Regency. Each stup took three samples of worker bees for morphometric analysis (n=120). Morphological characters were observed under a stereo microscope connected to an Optilab camera. The character of *T. clypearis* is the thorax is black and hairy, and there are six hair bands on the mesoscutum and dark brown tibia. Metasoma has a variety of colours, ranging from pale yellow with black stripes, yellow and black, and pale yellow. The morphometric measurements included body length, head width, wing length, genna width, second flagellomere diameter, and body weight. The results showed that the seven morphometric measurement variables of the worker bee *Tetragonula clypearis* did not show significant differences ($P>0.05$) in the two cultivation groups. The value of the diversity coefficient $<15\%$ indicates that the morphometrics of the worker bee *Tetragonula clypearis* is uniform in both cultivation groups in Salut Village, District, Kayangan, North Lombok Regency.

Keywords: diversity; *tetragonula clypearis*; worker bees; morphological morphometric

Introduction

Due to the variety of stinging and stingless honey bee species found there, Indonesia is renowned worldwide as a honey bee country (Suranto, 2005). Stingless honey bees play a crucial part in producing honey, propolis, bee bread, and bee wax, all of which have significant economic value and are healthy in addition to being potential pollinators for improving the growth of diverse plants (Kumar et al., 2012).

More than 600 stingless bees have the highest diversity of corbiculate bees (Eardley, 2004; Michener, 2007; Moure, 1961; Rasmussen, 2008). These bees are found in extensive tropical forests, farms, and urban areas throughout the Neotropical, Afrotropical, and Australasian regions (Michener, 2007). The Indonesian archipelagos of Sumatra, Java,

Timor, Borneo, Sulawesi, Ambon, Maluku, and Irian Jaya contain 46 known stingless bee species as a member of the Indo-Malayan and Australasian ecoregions (Kahono et al., 2018; Olson et al., 2001). However, the diversity of stingless bees in North Lombok Regency has not been widely studied. Therefore, observing the morphometric identification of stingless bees in the North Lombok Regency is necessary.

The exterior characteristics of workers are used for the morphological identification of stingless bees (Moure, 1961; Sakagami, 1978; Sakagami et al., 1990). *Tetragonula* is a genus of little stingless bees, according to Sakagami (1978), who also noted intra-specific variations in these body characteristics. So it's crucial to document additional phenotypic traits, such as nest structure, to help with species identification (Wille & Michener, 1973).

Tetragonula clypearis is a species of stingless bee commonly found in Indonesia. *T. clypearis* usually nests in tree trunks about 2-4 m from the ground. The entrance to the nest is a tube surrounded by black resin. The mouth of the hive can be an internal and external tube for bees to enter and exit the hive (Chinh et al., 2005). This study aims to describe one species of a worker bee, *Tetragonula clypearis*, from Salut Village, Kayangan District, North Lombok Regency, based on morphometric characters.

Materials and Methods

Location

The research location is in two Honeybee Cultivation Livestock Groups, namely the Harapan Keluarga Group and the Tunas Muda Group in Salut Village, Kayangan District, North Lombok Regency. The morphometric analysis was carried out at the Laboratory of Animal Breeding and Genetics, Faculty of Animal Husbandry, University of Mataram.

Total sample

Bee samples were collected from 40 stup colonies of *T. clypearis* bees from each livestock group, 20 stup in the Harapan Keluarga group, and 20 stup in the Tunas Muda group. Three samples of worker bees were taken from each stup for morphometric analysis (n=120). The bee sampling method used the handling method, namely the collection of bees using a plastic bottle placed in the mouth of the beehive. Furthermore, the bees were stored in a 2 ml tube containing absolute ethanol.

Bee Dry Sample Preservation

The bee sample was stored in a 2 ml tube containing absolute ethanol and then immersed in hot water for several minutes to loosen the rigid bee sample, making it easy to adjust the bee's body parts. Then the bee sample was pinned with an insect needle measuring 00 (0.30 mm diameter). The needle is inserted in the dorsal or lateral aspect of the bee's thorax (Borrer et al., 1989). The pinning process serves for preservation or to facilitate observation under a microscope.

Observation of Worker Bee Morphology

The morphology observed was mainly body colour, thorax colour, mesonotum, scutellum, middle posterior of propodeum, abdomen, forewing colour, hamuli, and posterior fringe of hind tibia; each part is described descriptively in the key identification of specific species found in North Lombok Regency. Morphological characters were observed under a stereo microscope connected to an Optilab camera. Identification of bee specimens was carried out by matching morphological and morphometric characters based on Schwarz (1939), Sakagami (1978), Dollin et al. (2015), and Smit (2012). The morphometric characteristics measured were body length, thorax width, front wing length, rear wing length, genna width, the diameter of the second flagellomere, and body weight (Sakagami et al., 1990; Smith, 2012).

Data analysis

The results of the measurement of morphometric characters were calculated as the average, standard deviation, and coefficient of diversity using Microsoft Excel 2019 and compared with the results of other studies.

Results and Discussion

The morphology of the worker bee *T. clypearis*

The identification results of 40 stup bee colonies collected from two groups of beekeeping in North Lombok Regency showed that all colonies belonged to the genus *Tetragonula* with the species *T. clypearis*, which was indicated by its main characteristics, namely, wings having five hamuli, mandibles having two teeth, and mesoscutellum extends beyond the propodeum (Sakagami, 1978). *Tetragonula* is a genus with a wide distribution in the Indo-Pacific region covering Asia and Sri Lanka (Sakagami 1978), Southeast Asia (Inoue et al., 1984), the Philippines (Sakagami, 1978; Schwarz, 1939), Malaysia (Kelly et al., 2014; Salim et al., 2012; Schwarz, 1939), Australia (Dollin et al., 2015), Thailand (Boontop et al., 2008), and India (Rathor et al., 2013). one of 10 genera comprised 46 species of propolis bees in Indonesia. Species of the genus *Tetragonula* have been found in

Sulawesi (Schwarz, 1939), East Kalimantan (Syafrizal et al., 2012), Sumatra West Sumatra (Putra et al., 2016), Bali (Putra et al., 2016), Java, Kalimantan, Ambon, Maluku and Papua (Schwarz, 1939; Kahono et al., 2018).

The morphological characteristics observed in the worker caste of the species *T. clypearis* (Figure 1A) included a black and hairy thorax and six hair bands on the mesoscutum and dark brown tibia. Metasoma has various colours,

namely pale yellow with black stripes, yellow and black like that of *T. fuscobalteata*; some have a pale yellow colour, such as *T. Minangkabau* (Schwarz, 1939; Sakagami, 1978; Dollin et al., 2015). The honeycomb structure of *T. clypearis* can be seen in Figure 1B. Morphological characteristics, Nest part, and Brood cells of *T. clypearis* based on the identification key of the Indo-Malayan stingless bee are presented in Table 1.

Table 1. Morphological characteristics, Nest part, and Brood cells of *T. clypearis* based on the identification key of Indo-Malayan stingless bee

No	Characters	Nest part	Brood cells
1	Body color: Bicolourous	Opening shape: Irregular, Oval, and Ellipse	Arrangement: Semi-comb
2	Thorax: Black	Opening diameter/ width: 2.5±1.2 cm	Shape: Sub-spherical
3	Mesonotum: six longitudinal hair bands, including the lateral fringes, are present.	Opening height: 2.1 ± 0.8 cm	Diameter: 0.24 ± 0.01 cm
4	Scutellum: Extended over the propodeum	External entrance length: 2.6 ± 0.9 cm	Colour: Light to dark brown
5	Middle of the propodeum's posterior: a smooth, shining area without hair	Shape: Funnel	Pillars/Connectives: Columnar (0.2 ± 0.1 cm) and lamellate
6	Abdomen: Tergite 1-2 yellowish to dull brown, 3-6 bright to dark brown	Ornamentation: Irregular ridges and lamellate propolis	
7	Forewing color: Clear	Colour: Blackish brown, dark brown, yellowish	
8	Hamuli: Five		
9	Posterior fringe of the hind tibia: Pulmose (branched)		

Morphological characteristics: Smith, 2012 and Dollin et al., 2015; Nest part and Brood cells: Wille & Michener (1973)

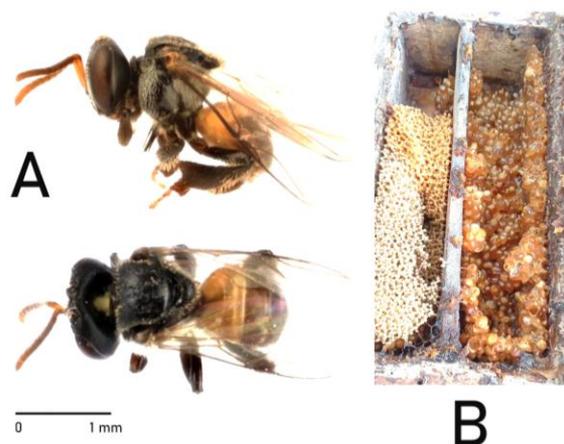


Figure 1. (A) *T. clypearis* bee; (B) nest part and brood cells *T. clypearis*

Morphometrics of the Worker Bee *T. clypearis*

The results of measurements of 120 individual worker bees of *T. clypearis* from two groups of bees in Salut Village, Kayangan

District, North Lombok Regency, are presented in Table 2.

The results of measurements of body sizes of *T. clypearis* bees in two groups of bees in Salute Village, Kayangan District, North Lombok Regency, were not statistically significant ($P > 0.05$). The coefficient of diversity (CD) values ranged from 1.262% - 5.903%; this indicates that the morphometric diversity of the worker bee *T. clypearis* is in a low category or close to uniformity. Kurnianto (2009) states that the diversity category is divided into three, namely small diversity ($CD \leq 5\%$), moderate diversity ($5\% < CD < 15\%$), and high diversity ($CD \geq 15\%$). The morphological characteristics and morphometric measures of the stingless bee specimens were determined using the descriptions provided by Smith (2012), Syafrizal et al. (2020), Trianto & Purwanto (2020), Purwanto & Trianto (2021), and Sayusti et al. (2021).

Table 2. Morphometric characters of worker bees of *T. clypearis* from Salut Village, Kayangan District, North Lombok Regency

No	<i>T. clypearis</i> body character	Location 1		Location 2		Mean of two locations
		Mean	CD (%)	Mean	CD (%)	
1	Body Length (mm)	3,019 ± 0,043	1,433	3,007 ± 0,055	1,848	3,013±0,049
2	Head Width (mm)	1,382 ± 0,047	4,584	1,511 ± 0,029	2,835	1,446±0,038
3	Front Wing Length (mm)	2,977 ± 0,116	3,909	2,979 ± 0,085	2,856	2,978±0,104
4	Rear Wing Length (mm)	2,104 ± 0,124	5,903	2,065 ± 0,081	3,941	2,085±0,102
5	Genna Width (mm)	0,218 ± 0,014	4,685	0,211 ± 0,010	3,201	0,214±0,012
6	Diameter of the second Flagellomere	0,143 ± 0,002	1,672	0,142 ± 0,001	1,262	0,143±0,002
7	Body Weight (g)	0,002 ± 0,000	3,448	0,002 ± 0,000	3,225	0,002±0,000

CD: coefficient of diversity; Location 1: Harapan Keluarga group; Location 2: Tunas Muda group

Body Length

T. clypearis is a species that has the smallest body size compared to *T. sapiens* and *T. biroi*. This opinion is supported by Heard (2016), which states that *T. clypearis* is a bee with a small size compared to *T. sapiens*. *T. clypearis* has a body length of 3,013±0,049 mm. This body size is not much different from that found in Australia, which is 2,3 – 3,7 mm (Dollin et al., 2015). The results of the study by Salatnaya

(2021) reported that *T. clypearis* had a body length of 3,16±0,19 mm, *T. sapiens* was found to be 3,91±0,06 mm, and *T. biroi* had a body length of 4,74±0,21 mm. Anaktototy (2020) found the body length of the bees *T. Fuscobalteata* 3.06±0.28 mm and *T. Sapiens* 3.67±0.11 mm.

The morphometric difference in body length is one form of bee adaptation to the environment (Gaston et al., 2008), which can be caused by differences in location and food sources (Ab Hamid et al., 2016). Loading plot

analysis shows that the body lengths of *T. clypearis*, *T. sapiens*, and *T. biroi* bees have a negative correlation because they are in different quadrants (Salatnaya, 2021).

T. sapiens is a bee with large body morphology, while *T. clypearis* is a species with small body morphology. *T. biroi* is a species with a body length more significant than the two and is a marker of morphological characters to distinguish the three species (Salatnaya, 2021).

Head and Genna Width

The mean head width of *T. clypearis* worker bees at locations 1 and 2 was 1.446 ± 0.038 mm. Head width measurement results in this study were not significantly different from the study of Salatnaya (2021), which reported that the head width of the *T. clypearis* bee was 1.54 ± 0.02 mm, while the head width of *T. sapiens* and *T. biroi* bees is 1.85 ± 0.01 mm. Anaktototy (2020) stated that the head width of the bee *T. Fuscobalteata* was 1.39 ± 0.08 mm and *T. Sapiens* 1.81 ± 0.05 mm.

The genna width of the *T. clypearis* worker bee at both study sites was 0.214 ± 0.012 mm. Salatnaya (2021) found that the genna width of *T. clypearis* was 0.20 ± 0.02 mm, *T. sapiens* was 0.25 ± 0.01 mm, and *T. biroi* 0.23 ± 0.00 mm. The results of genna width measurements in the Anaktototy study (2020) in *T. Fuscobalteata* bees were 0.22 ± 0.03 mm and *T. Sapiens* 0.29 ± 0.05 mm. The results of previous studies showed no significant differences in the genna width of *T. clypearis*, *T. sapiens*, *T. biroi*, and *T. Fuscobalteata* bees.

Wing Length

In Table 1, the results show that the average forewing length of the *T. clypearis* worker bee at both locations was $2,978 \pm 0.104$ mm and the hindwing length of the *T. clypearis* worker bee was 2.085 ± 0.102 mm. The forewings of the *T. clypearis* bee are longer than the hind wings. The results of the forewing length measurements were slightly different from those of Salatnaya (2021) on *T. clypearis* bees from West Halmahera Regency, 3.25 ± 0.18 mm. While the forewings of *T. sapiens* and *T. biroi* bees were 4.13 ± 0.02 mm and 4.41 ± 0.02 mm, respectively. Anaktototy (2020) measured the

forewing length of *T. fuscobalteata* bees at 3.19 ± 0.21 mm and *T. Sapiens* at 3.88 ± 0.07 mm. The hind wings of *T. fuscobalteata* were 2.22 ± 0.11 mm, and *T. Sapiens* were 2.72 ± 0.30 mm.

The front wing carries out manoeuvres, flies fast, and carries heavy loads (Sihombing, 2005). Different temperatures and wind speeds influence the difference in wing length in bees. Different altitudes will have different wind speeds and temperatures. This supports Dwiyono (2014) claim that highland bees have longer hind wings than lowland bees; it is hypothesized that this adaptation to wind speed in the highlands has caused the bees' wing size. Meanwhile, according to Bertoni (2013), the distance travelled, and the presence of plants that are a source of nectar in each location affect the difference in the length and width of the worker bees' hind wings.

Diameter of the second Flagellomere

The mean diameter of the second flagellomere of *T. clypearis* worker bees at locations 1 and 2 was 0.143 ± 0.002 mm. Salatnaya (2021) found that the diameter of the second flagellomere *T. clypearis* was 0.13 ± 0.00 mm, *T. sapiens* was 0.15 ± 0.00 mm, and *T. biroi* was 0.15 ± 0.00 mm. Measurement results of the Diameter of the second Flagellomere in the Anaktototy study (2020) on *T. Fuscobalteata* bees were 0.10 ± 0.01 mm and *T. Sapiens* 0.13 ± 0.01 mm. The results of previous studies showed no significant differences in the diameter of second flagellomere measurements of *T. clypearis*, *T. sapiens*, *T. biroi*, and *T. Fuscobalteata* bees.

Flagellomere segments in *Tetragonula* sp bees are generally brown and consist of 10 parts. While the antenna size is shorter than the length of the head, and metasoma has 3-7 flagellomere segments.

Body Weight

The average body weight of *T. clypearis* worker bees at locations 1 and 2 was 0.002 ± 0.000 g. *T. laeviceps*, *L. terminata*, and *H. itama* had average fresh body weights of 0.002 g, 0.005 g, and 0.010 g, respectively (Pangestika et al., 2017). The results showed that the body weights

of *T. clypearis* and *T. laeviceps* had the same body weight and were smaller than *L. terminate* and *H. itama*.

According to Ruttner (1988), adaptation to environmental conditions reflects changes in the size of worker bees. According to Roubik & Ackerman (1987), the worker body size for social bees is typically regarded as an adaptation foraging and taking advantage of flower resources. Body size dramatically affects the flight distance of bees looking for food. The bee's increased body size was matched by its larger corbicula, which carried more pollen while foraging (Veiga et al., 2013).

The highest pollen load was recorded by *H. itama* (31392 pollen grains), while the lowest pollen load was recorded by *T. laeviceps* (8015 pollen grains). The earlier research by Reinaldo (2015) discovered that *T. laeviceps* had lower pollen loads than *Apis cerana* (4228 pollen grains). The size of the pollen basket and the worker bees' body size affect their capacity to carry pollen (Sadeh et al., 2007; Shuel, 1992). Young colony members use the protein found in pollen (Jalil, 2014). Weight and the quantity of pollen on stingless bees' bodies were positively connected (Pangestika et al., 2017).

Because of their tiny bodies, these bees are known as general visitors of blooming plants in the tropics because they can move more swiftly to access a variety of blooms (Trianto & Purwanto, 2020; Witter et al., 2014). Body size dramatically affects the flight distance of bees looking for food. The bigger the bee's body, the farther the flight distance. The bee *Tetragonula sp.*, which is 5 cm, has a flight distance of about

600 m (Amano et al., 2000). According to Ruttner (1988), adaptation to environmental conditions reflects changes in the size of worker bees. Roubik and Ackerman (1987) add that body size has generally been considered an adaptation to activities for foraging and exploiting interest resources for a more social worker.

Morphometric comparison of *T. clypearis*

The morphometric comparison of *T. clypearis* with previous studies is presented in Table 3. The morphometric comparison of *T. clypearis* with several *Tetragonula* species is presented in Table 4. The morphometric differences of *T. clypearis* at different locations can be seen in body and wing length (Table 3). The results of the reference search using the Publish or Perish application version 8 show that the morphometric research of *T. clypearis* indexed by Scopus only found one publication, namely the results of the study of Sayusti et al. (2021). This shows that research on morphometric *T. clypearis* is still very little.

The morphometric comparison of *T. clypearis* with previous studies based on different *Tetragonula* species showed that body length and wing length also had significant differences. Head Width, genna width and diameter of the second flagellomere are no different. The results of the morphometric comparison also showed that *T. clypearis* had a smaller body size than *T. laeviceps*, *T. sapiens*, *T. biroi*, *T. iridipennis* and *T. sarawakensis*.

Table 3. Comparison of *T. clypearis* morphometrics with previous studies based on different locations

No	Morphometric of <i>T. clypearis</i>	* North Lombok	** West Halmahera	***South and West Sulawesi
1	Body Length (mm)	3,013±0,049	3,16±0,19	3.6±0.2
2	Head Width (mm)	1,446±0,038	1.54±0.02	-
3	Front Wing Length (mm)	2,978±0,104	3.25 ± 0.18	-
4	Rear Wing Length (mm)	2,085±0,102	-	-
5	Genna Width (mm)	0,214±0,012	0.20±0.02	0.269±0.085
6	Diameter of the second Flagellomere (mm)	0,143±0,002	0.13±0.00	0.131±0.006
7	Body Weight (g)	0,002±0,000	-	-

*: This study; **: Salatnaya (2021); ***: Sayusti et al. (2020)

Table 4. Morphometric comparison of *T. clypearis* with previous studies based on different *Tetragonula* species

No	Morphometric Characteristic	¹ T. clypearis	² T. laeviceps	³ T. sapiens	³ T. biroi	³ T. iridipennis	³ T. sarawakensis
1	Body Length (mm)	3,013	3.92	3.76	4.00	3.74	4.60
2	Head Width (mm)	1,446	1.64	1.81	1.84	1.57	2.10
3	Front Wing Length (mm)	2,978	3.59	3.91	3.83	3.74	4.55
4	Rear Wing Length (mm)	2,085	2.44	2.70	2.37	2.71	3.28
5	Genna Width (mm)	0,214	0.24	0.24	0.26	0.24	0.37
6	Diameter of Flagellomere (mm)	0,143	0.15	0.14	0.14	0.14	0.16
7	Body Weight (g)	0,002	-	-	-	-	-

¹: This study; ²: Rachmawati et al. (2022); ³: Trianto and Purwanto (2020)

Conclusion

Tetragonula clypearis is the most commonly found species and is evenly distributed in North Lombok Regency. The distinctive character of *T. clypearis* is the thorax is black and hairy, and there are six hair bands on the mesoscutum and dark brown tibia. Metasoma has a variety of colours, ranging from pale yellow with black stripes, yellow and black, and pale yellow. The seven morphometric measurement variables of the worker bee *Tetragonula clypearis* did not show significant differences ($P > 0.05$) in the two cultivation groups. The value of the diversity coefficient $< 15\%$ indicates that the morphometrics of the worker bee *Tetragonula clypearis* is uniform in both cultivation groups in Salut Village, District, Kayangan, North Lombok Regency.

Acknowledgements

We would like to thank our stingless bee farmers: the Harapan Keluarga group and the Tunas Muda group, for providing samples and information during this study.

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